Reference: *Biol. Bull.* **176:** 176–190. (April, 1989)

Mechanisms of Cardiovascular Compensation for Gravity in Bluefish (*Pomatomus saltatrix*)

CHRISTOPHER S. OGILVY*, STEPHEN H. FOX**, AND ARTHUR B. DUBOIS

John B. Pierce Foundation Laboratory, New Haven, Connecticut 06519

Abstract. Circulatory tolerance for gravity was examined in anesthetized and unanesthetized bluefish (*Pomatomus saltatrix*) tilted head up in air for short or prolonged periods. Blood pressure (BP) and heart rate (HR) were monitored using a ventral aortic catheter. During tilts in unanesthetized fish, BP decreased slightly, and tachycardia occurred. Anesthesia lowered resting BP while the response to tilting was similar to unanesthetized fish. Spinal cord transection 3 mm caudal to the obex was used to interrupt sympathetic outflow and paralyze the lower body. It produced a reduction and subsequent fall in BP during tilting. Spinal cord transection combined with vagotomy produced pronounced tachycardia, with a precipitous fall in BP with stable HR during tilting. Phentolamine mesylate (400 μg/kg) produced a fall in control BP and further reduction during tilting. When 80 μg/kg of atropine sulphate was given, tachycardia occurred and BP fell during tilting yet not below levels measured in control fish. Transient elevations of BP associated with body contractions during tilting were abolished with pancuronium bromide. Circulatory tolerance of bluefish for gravity depends upon integrity of the spinal cord and sympathetic innervation of peripheral vasculature as well as intact parasymathetic cardiac innervation. Muscular contraction of the lower body also contributes to maintaining BP during tilts.

Introduction

The cardiovascular changes that occur in humans and other mammals in response to passive head-up tilting have been the subject of many studies (see Blomquist and Stone, 1983, for review). There is typically a tachycardia with an associated fall in pulse pressure and cardiac output, and increased systemic vascular resistance. Mean blood pressure remains stable or slightly elevated. These reflex responses are thought to be mediated by afferent input from peripheral pressure and chemoreceptors and subsequent appropriate output from central autonomic cardiovascular control centers (Abboud and Thames, 1983). The overall response acts to maintain blood pressure and thereby continue perfusion to the brain, kidney, liver, and other vital organs.

In that fish live immersed in water, it may not be expected that they should have or need cardiovascular reflex responses to gravity similar to mammals. However, in earlier studies we found that bluefish (a teleost, *Pomatomus saltatrix*) were able to tolerate the physiologic stress of head-up tilting in air with only slight reduction in arterial blood pressure whereas a species of chondrichthyes (smooth dogfish, *Mustelus canis*) could not (Ogilvy and DuBois, 1982). One reason bluefish were able to avoid the orthostatic hypotension, narrowing of pulse pressure, and caudal peduncle edema observed in dogfish was thought to be the existence of a more highly organized and well-developed autonomic cardiovascular reflex system. Indeed, recent work in other laboratories has documented well-developed sympathetic and parasympathetic response systems in teleost fish which are important in maintaining vasomotor tone at rest (Smith *et al.*, 1985) and mediating cardiovascular changes during swimming (Axelsson and Nilsson, 1986; Axelsson, 1988; Priede, 1974). The purpose of the present study was to further evaluate the presence and contribution of sympathetic and parasympathetic reflexes as well as lower body muscle tone in the cardiovascular response to head-up tilting in bluefish. To do this, we used selective pharmacologic blocking agents and surgical lesions to remove one or more of the sympathetic, parasympathetic, or muscular tone components of the response.

Received 21 March 1988; accepted 24 January 1989.

* Present address: Neurosurgical Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

** Present address: Ithaca College, Ithaca, NY 14850.

Materials and Methods

General handling of fish

Bluefish were caught with a hook and line in the waters around Woods Hole and kept in a tank with running salt water until used one to six days later. They were transported individually to a nearby laboratory in a small tank containing 40 l of seawater with 2 g of tricaine methanesulfonate (Aldrich Chemical Co., Milwaukee, WI). In one set of experiments no anesthetic was used. Each fish was weighed and then placed on a foam rubber pad on a V-board that was supported on a wooden trough containing a 50:50 dilution of the anesthetic solution with seawater. A small pump located in the trough pumped water through a rubber tube to a Y-tube inserted in the mouth of the fish which delivered running water to the gills. A dark piece of polyethylene was placed over the eyes of the fish to minimize visual stimuli.

Blood pressure in the ventral aorta was measured using a technique described in previous experiments (Ogilvy and DuBois, 1982). Briefly, an 18 gauge thin walled spinal fluid needle (18-T) was inserted through the floor of the mouth between the third and fourth gill arches. The obturator was removed and blood flow out of the needle confirmed adequate position in the ventral aorta. A 1-mm polyethylene tube (PE 50) was inserted into the aorta through the spinal needle. The needle was then removed with the PE 50 tubing left 4 to 5 cm into the aorta from the point of entry through the floor of the mouth. This distance placed the catheter tip 5 to 8 mm distal to the aortic valve. The PE 50 tubing was then sutured to the floor of the mouth to prevent movement during the experiment. The 1.0 meter long catheter was connected to a strain gauge manometer (Gould-Statham P23ID) to measure blood pressure. The aortic catheter was flushed intermittently with small amounts of Forster-Taggart teleost Ringer's solution containing sodium heparin (Elkins-Sinn) 20 units per ml.

The blood pressure manometer was balanced with a zero reference level at the height of the catheter tip in the ventral aorta and was calibrated with a water column. Blood pressures are expressed in mmHg. A second manometer was connected to a tube mounted on the V-board and it measured the pressure (also expressed in torr) in a column of water whose surface was at the height of the ventral aorta. Therefore, during tilting we always referred the pressures to a zero reference level at the height of the ventral aorta. Pressures were recorded continuously throughout the experiments on a direct-writing polygraph (model 5, Grass Instrument Co., Quincy, Massachusetts). Speed of response of the catheter and gauge system was measured by removing a finger tip from the end of the liquid-filled PE-50 tubing. The pressure response was 64% complete in 0.026 s, and 90% complete in 0.040 s.

Table I

Blood pressure (systolic/diastolic, mmHg) and heart rate (bpm) of resting bluefish before and after immersion in water

	In air			In water		
Fish #	Condition	P_{va} mmHg	HR bpm	Condition	P_{va} mmHg	HR bpm
21 (1975)	V-board tricaine	91/59	36	pool seawater	94/65	72
23 (1975)	V-board tricaine	59/26	30	tank seawater	53/26	30
13 (1983)	V-board no anesthesia	78/68	56	trough no anesthesia	82/68	54
Mean		76/51	41		76/53	52

Resting values in air versus water

To compare blood pressure and heart rate of bluefish in water with values obtained resting on a V-board in air, we reviewed experiments that had been done in previous years. The three in which conditions were most comparable in air and in water are as follows. The mean in air was 76/51 mmHg with a heart rate of 41 bpm and in water was 76/53 mmHg with a heart rate of 52 bpm (Table I). In air the blood pressure gauge had its reference level set at the height of the ventral aorta, whereas when the fish was in water the gauge was rebalanced to a zero reference level at the water's surface. We found that bluefish placed under water had the same average blood pressure and heart rate as bluefish on a V-board in air provided the physiological state had not changed

Tilting protocols

Two separate tilting protocols were used in this study. In order to evaluate the blood pressure and heart rate changes to short periods of tilting, bluefish were tilted 10°, 20°, and 30° head up for 2 min, 2 min, and 5 min, respectively. This series of tilts was conducted in one group of unanesthetized fish (n = 6) as well as in a group of anesthetized fish (n = 5) in which the series of short duration tilts was repeated following the intra-arterial administration of phentolamine mesylate (400 μg/kg).

Longer duration tilts were conducted only in anesthetized fish. To establish a baseline response to 30-min tilts in anesthetized fish, heart rate and blood pressure measurements were made in 8 "control" bluefish during tilting following ventral aortic cannulation as described above. A control period of 20 min was allowed to elapse prior to the tilt. Fish were then tilted head up for 30 min

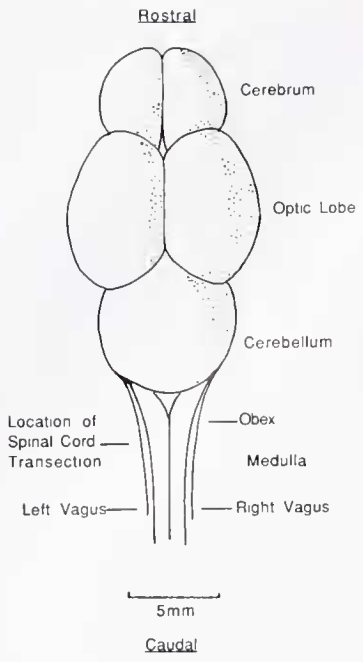

Figure 1. Dorsal view of bluefish brain, drawn from a photograph. A craniotomy exposed the cerebellum and upper cervical spinal cord. The placement of the spinal cord transection is indicated. When bilateral vagotomies were performed, the vagal fibers were transected at the same level as the spinal cord.

at an angle of 30°. A recovery period of 30 min was allowed after the tilting episode. Blood pressure and heart rate measurements were made from the polygraph record at regular intervals throughout the experiment.

To interrupt central descending sympathetic pathways and produce muscular flaccidity, we transected the spinal cord after placing the ventral aortic catheter as described above. To create spinal cord lesions, we removed the skin and musculature over the posterior one third of the skull. This exposed the central spine of the skull which was removed using an electric drill with a burr attachment (Dremel Moto-Tool, model 370-5, Racine, Wisconsin). A craniotomy was then performed using the burr attachment to carefully remove bone and expose the dura overlying the upper cervical region and lower medulla. The dura was then opened sharply and the spinal cord was exposed (Fig. 1). The vagal nerves run adjacent to the spinal cord at the level of our dissection and these fibers were carefully separated from the spinal cord using microsurgical instruments and magnification. After vagal fibers were clearly separated, the spinal cord was sharply transected using microscissors at a level 3 mm caudal to the obex (Fig. 1). Following spinal cord transection, the fish was left undisturbed for 20 min while blood pressure and heart rate were continuously monitored. The fish was then tilted head-up to 30° for 30 min. After returning to the horizontal position, blood pressure and heart rate were followed for 30 min as in fish without cord transection (control fish). In a separate set of 4 bluefish, vagal nerve fibers were transected in conjunction with the spinal cord at a level 3 mm caudal to the obex. These fish were then subjected to short tilts of 10°, 20°, or 30°.

Tilting after pharmacologic blockade

Two separate sets of experiments using pharmacologic blockade were conducted. To block alpha-adrenergic receptors, phentolamine mesylate was given intra-arterially at a dose of 400 μg/kg to each of 5 bluefish. Ten minutes were then allowed to elapse prior to a 30 min, 30° head-up tilt. As in previously described protocols, there was a 30-min recovery period after tilting. As noted above, a series of short duration tilts was also conducted in a separate set of fish after administration of phentolamine. The decision to use a dose of 400 μg/kg was based on a separate set of experiments (Fox *et al.*, 1988) where this dose of phentolamine was found to significantly alter the blood pressure response to graded doses of epinephrine.

Intra-arterial injection of atropine sulfate was used to block cardiovascular cholinergic effects in five bluefish. A dose of 80 μg/kg was used which produced a maximal heart rate (Fox *et al.*, 1988). Ten minutes after the administration of atropine, bluefish were tilted head-up for 30 min. As with phentolamine and control experiments, a follow-up period of 30 min was used.

Control experiments

In order to determine if the surgical dissection, without cord transection, had any influence upon cardiovascular function and response to tilting, we conducted a series of 2 to 5 min 10°, 20°, or 30° head-up tilts on bluefish prior to and after exposure of the spinal cord. In 4 bluefish, blood pressure and heart rate were followed with the fish on the V-board in the horizontal position for 80 min after spinal cord transection without vagotomy as described above (Table II).

Statistical analysis of data

Student's two tailed *t*-test is used to compare values of paired data for blood pressure or heart rate at each time interval during tilting to the control values recorded one min prior to tilting for the 30-min tilts. For short duration tilts, each time interval during tilting is compared to the value 10 s prior to tilting.

Results

Blood pressure and heart rate changes observed in 6 unanesthetized bluefish during head-up tilts of 10°, 20°,

Table II

Average blood pressure and heart rate before and after spinal cord transection in four anesthetized bluefish

	Time prior to transection (min)				Time after spinal cord transection (min)					
	20	10	5	1	5	10	20	40	60	80
Blood pressure (mm Hg)										
Systolic	83	76	82	84	65	68	59	54	54	56
SE	7.1	8.2	9.0	8.5	6.5	6.9	9.1	3.9	2.4	
Diastolic	53	53	54	54	47	45	43	39	37	42
SE	6.7	4.5	3.8	5.0	9.7	11.1	8.2	5.1	5.0	
Heart rate	48	53	47	44	51	42	53	42	35	48
SE	6.5	4.5	1.5	2.9	13.1	15.0	9.9	9.8	3.9	

and 30° are shown in Figure 2. There was a slight decrease in systolic and diastolic blood pressures during 10° and 20° tilts which was more pronounced during the 30° tilt. Heart rate increased during tilting, with higher angles of tilt associated with higher heart rates. The increase in rate was statistically significant during the 30° tilt (Fig. 2). A comparable set of experiments was conducted in five anesthetized bluefish (Fig. 3). Anesthetized fish as a group had a significantly lower control blood pressure compared to unanesthetized fish ($P < 0.05$ for both systolic and diastolic pressures). Resting heart rate was 58 ± 3.0 in unanesthetized and 42 ± 5.8 in anesthetized fish ($P < 0.05$). The fall in blood pressure was significant during the 30° tilt in anesthetized fish but the heart rate did not change significantly during this tilt (Fig. 3).

Thirty degree tilts for 30 min were conducted in 8 anesthetized fish (Fig. 4). The initial response was similar to that observed during 5 min, 30° tilts. After the initial 5 min of tilting, systolic and diastolic blood pressures rose and were maintained for the duration of the tilt. The blood pressure 10 min into the tilt was 76/53 mmHg which represents a 13% reduction in systolic and 10% reduction in diastolic pressure compared to the pressure 1 min prior to the tilt. A significant tachycardia developed during the first 5 min of tilting and persisted throughout the 30-min tilt.

Tilting after pharmacologic blockade

Intra-arterial administration of phentolamine mesylate in a dose of 400 μg/kg was used to block alpha-adrenergic receptors. This produced a significant reduction in resting systolic blood pressure within 5 min of administration (Fig. 5). Tilting head-up to 30° produced a significant reduction in systolic blood pressure, which recovered slightly towards the end of the tilting interval. The blood pressure 10 min into tilt was 56/43 mmHg which represents a 23% reduction in systolic and an 8% reduction in diastolic pressure compared to the blood pressure 1 min prior to tilt. Upon returning to the horizontal position after tilting, blood pressure recovered within 5 min to a mean level of 82/61 mmHg which was between the initial control blood pressure (94/55 mmHg) and the pressure 1 min prior to tilting (73/47 mmHg).

Short duration tilts of 10°, 20°, or 30° in phentolamine treated fish showed a response similar to the longer duration tilts with the greatest fall occuring in systolic blood pressure during tilting (Fig. 6). The fall in blood pressure during tilting was most pronounced for 30° tilts.

Administration through the aortic catheter of 80 μg/kg of atropine sulfate in 5 anesthetized fish produced a significant tachycardia ($P < 0.01$) within 1 min (Fig. 7) which persisted during and after tilting. This tachycardia was associated with a rise in both systolic and diastolic blood pressures when the fish were horizontal. Blood pressure fell significantly and remained low compared to pre-tilt values during the first 20 min of tilting with a slight recovery towards the end of tilting (Fig. 7). Upon returning fish to the horizontal position, blood pressure recovered within 1 min.

Tilting after spinal cord transections with and without vagotomy

Figure 8 details the response of blood pressure and heart rate prior to and after spinal cord transection with preservation of both vagal nerves (Fig. 1). Prior to transection the heart rate was 44 ± 3.9 beats/min. Five minutes after transection heart rate was 59 ± 15.5 beats/min; 20 min after transection heart rate fell to 31 ± 4.7 beats/min. Blood pressure fell from 86/59 mmHg 1 min prior to cord transection to 66/50 mmHg 20 min after transection. During the 30° head-up tilt, heart rate increased while blood pressure fell initially and continued to fall throughout the tilt (Fig. 8). During recovery after tilting,

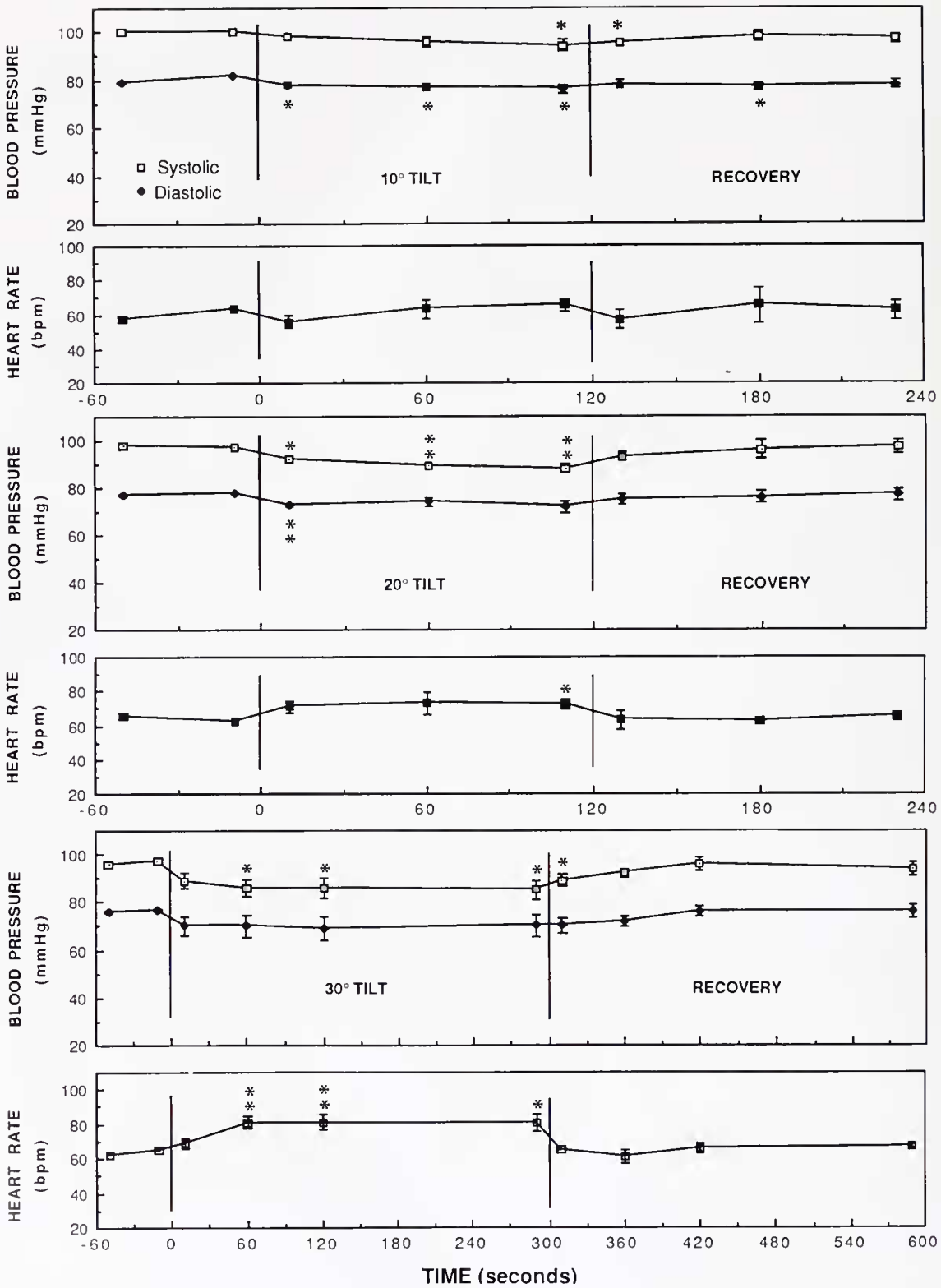

Figure 2. Response to head-up tilting in six bluefish without anesthesia. Ten and 20° tilts were maintained for 2 min, and the 30° tilt for 5 min. In this and subsequent figures the error bars represent ± one standard error of the mean using paired data to compare the values for each fish with its control value immediately before the tilt. One star indicates $P < 0.05$ and two stars $P < 0.01$.

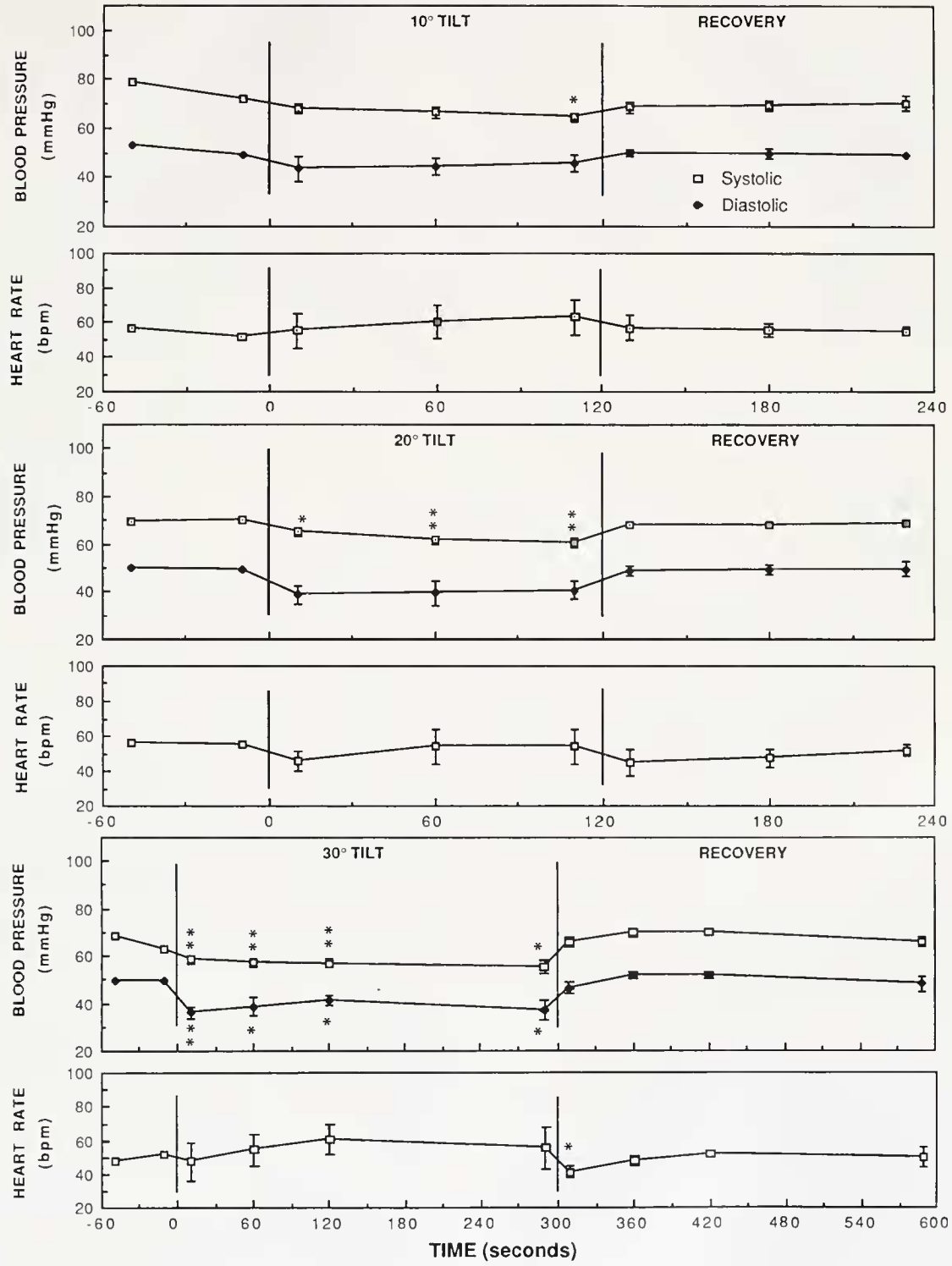

Figure 3. Heart rate and blood pressure response to 10° and 20° head-up tilts for 2 min or a 30° tilt for 5 min in 5 anesthetized bluefish.

blood pressure remained significantly lower than the pressure 1 min prior to tilt until 20 min after being returned to the horizontal position (Fig. 8).

Spinal cord transection was combined with vagotomy in four bluefish. Following this lesion, the average heart rate increased significantly and was associated with a fall

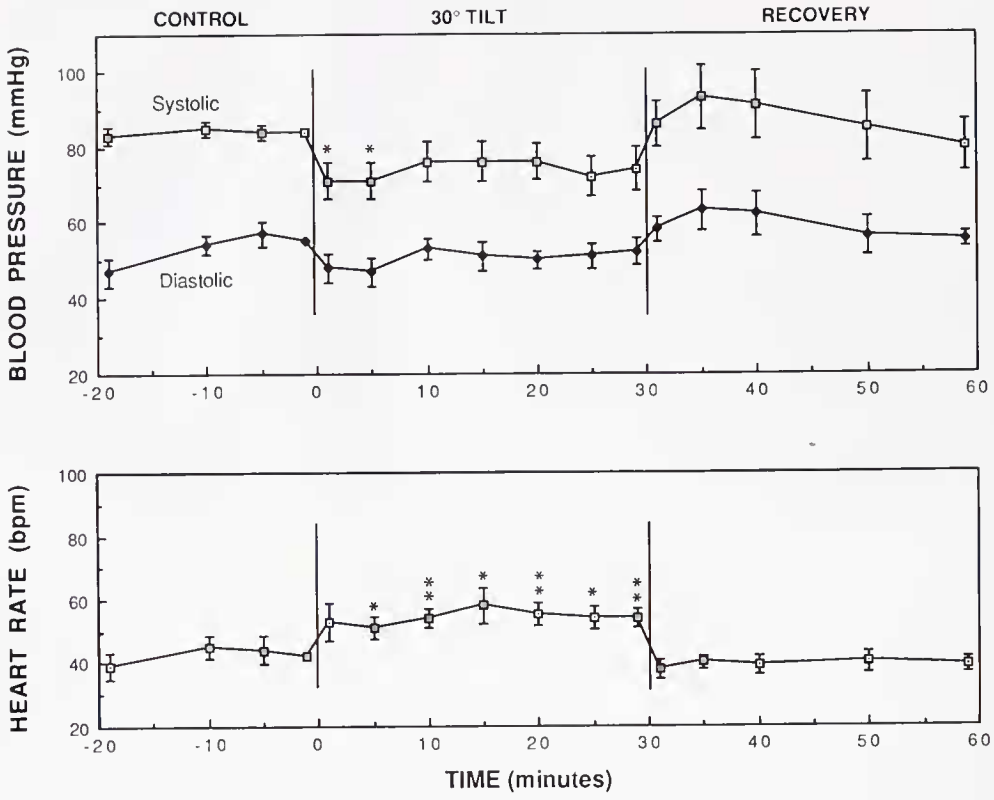

Figure 4. Blood pressure and heart rate changes before (control), during 30° tilt for 30 min, and throughout a 30-min recovery period in 8 anesthetized bluefish.

in pulse pressure as well as a slight reduction in resting mean blood pressure (Fig. 9). During tilts there was an immediate and precipitous drop in blood pressure. The decrease was greater during higher angle tilts. Pressure increased back to pre-tilt values within 10 s of returning these fish to the horizontal position. The heart rate was elevated after transection and did not increase further during tilting.

Behavioral influence on blood pressure

While bluefish rested in the horizontal position on the V-board, there were spontaneous variations in heart rate and blood pressure. When manipulations were made within the mouth there were often bradycardic episodes lasting 5–10 s. Occasional body movements consisting of alternating contractions of the body musculature were usually followed by slight (2–8 mmHg) increases in blood pressure in the ventral aorta. During head-up tilts these body movements became more frequent and often fish would exhibit 3–6 violent tail flaps that were followed by more significant increases in blood pressure. This was often true in tilts following administration of phentolamine when blood pressure was low prior to tilting. A typical example of blood pressure response to body movement is shown in Figure 10. Administration of the neuromuscular blocking agent pancuronium bromide (Elkin-Sinn, Inc.), 0.1 mg/kg intraarterially, to this fish abolished the body movements and the transient increases in blood pressure associated with body movements during tilting.

Control experiments

The response of blood pressure and heart rate to 10°, 20°, or 30° head-up tilting was the same prior to and after dissection to expose (yet not transect) the spinal cord. This analysis was conducted in 9 bluefish and the response was similar to that shown in Figure 3. Blood pressure and heart rate changes observed after spinal cord transection (no vagotomy) without subsequent tilting are shown in Table II. Blood pressure fell significantly from 84/54 mmHg 1 min prior to transection to 59/43 mmHg 20 min after transection. Between 20 and 60 min after spinal cord transection, the blood pressure remained relatively stable. The ultimate demise in 3 of the 4 fish in Table II was due to brady-arrhythmias occurring 60 to 80 min after transection. This was characterized by 2–4 s pauses in heart rate followed by resumption of the usual rate. These pauses became increasingly frequent

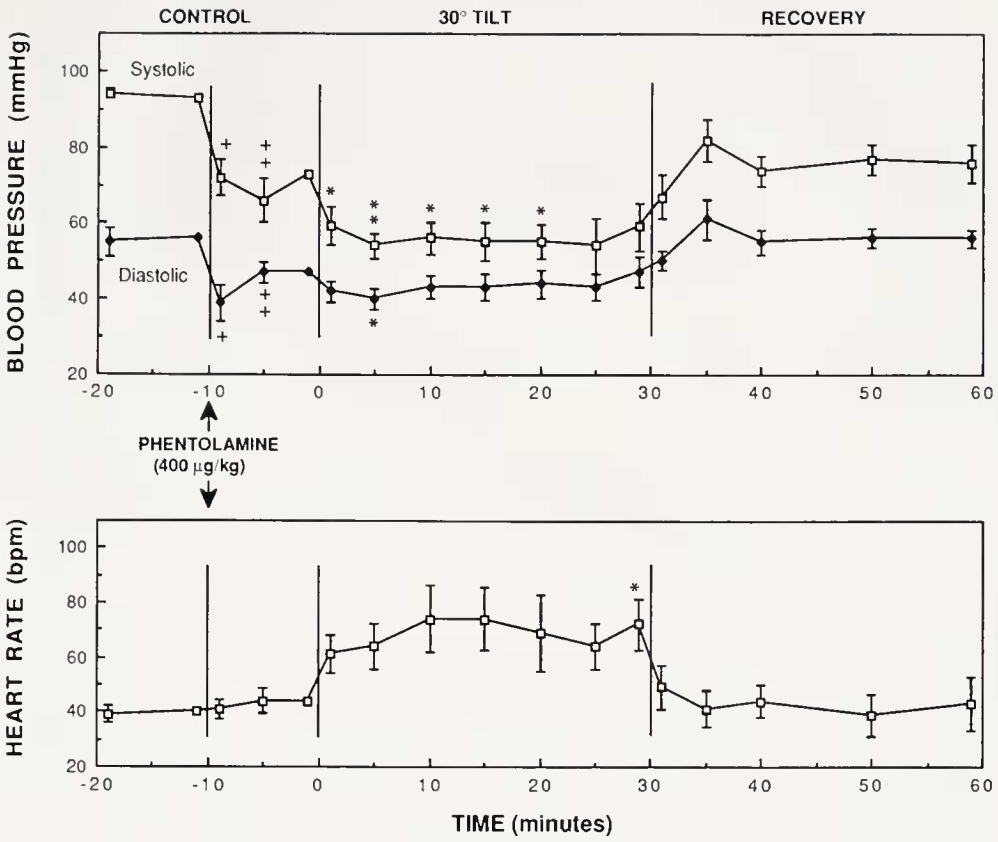

Figure 5. Phentolamine mesylate (400 μg/kg) was given in the ventral aorta 10 min prior to the 30 min, 30° head-up tilt in 5 anesthetized bluefish. In Figures 5 and 7 the single plus sign indicates $P < 0.05$ and double plus $P < 0.01$ for each fish relative to the pre-drug, or in Figure 8 pre-transection, control. The standard error bars after the drug or transection but before the tilting are paired data related to the pre-treatment control values.

over several minutes until ultimately permanent cardiac standstill occurred. These pauses were nonexistent in one bluefish in which the spinal cord was transected (vagi left intact) and 80 μg/kg of intraarterial atropine sulfate was given 10 min after transection. This fish exhibited the typical tachycardia observed in other fish following administration of this dose of atropine.

Discussion

The fact that bluefish can maintain blood pressure during head-up tilts has been documented in a previous study (Ogilvy and DuBois, 1982). However, the exact mechanism mediating this response has not previously been elucidated. In the present investigation we were able to examine the blood pressure and heart rate response during head-up tilts in unanesthetized fish, anesthetized fish, and in fish pretreated with various pharmacologic blockers or those subjected to selective or combined surgical lesions.

Unanesthetized bluefish had the highest resting blood pressure of any group studied. In addition, the resting heart rate in these fish of 55 to 65 beats/min (Fig. 2) was higher than control values of 40 to 55 beats/min observed in groups of anesthetized fish (Figs. 3, 4, 5, 7, 8). Indeed, certain general anesthetics are known to lower blood pressure and heart rate in mammals (Marshall and Wollman, 1985). The anesthetic used in our experiments does appear to have a similar effect on baseline levels of blood pressure and heart rate. This is in keeping with the findings of Houston *et al.* (1971), who documented a fall in dorsal aortic blood pressure following anesthetization of trout with 100 mg/l of tricaine methanesulphonate. Smith *et al.* (1985) found that mean ventral aortic blood pressure decreased from 5.3 kPa to 3.4 kPa while heart rate changed relatively little from 48 to 44 beats/min over 24 h after recovery from anesthesia with tricaine and surgical manipulations in codfish. It may be that despite our attempts to reduce stressful external stimuli, unanesthetized bluefish had higher degrees of sympathetic output to the heart and peripheral vasculature. However, these fish demonstrated occasional pauses of

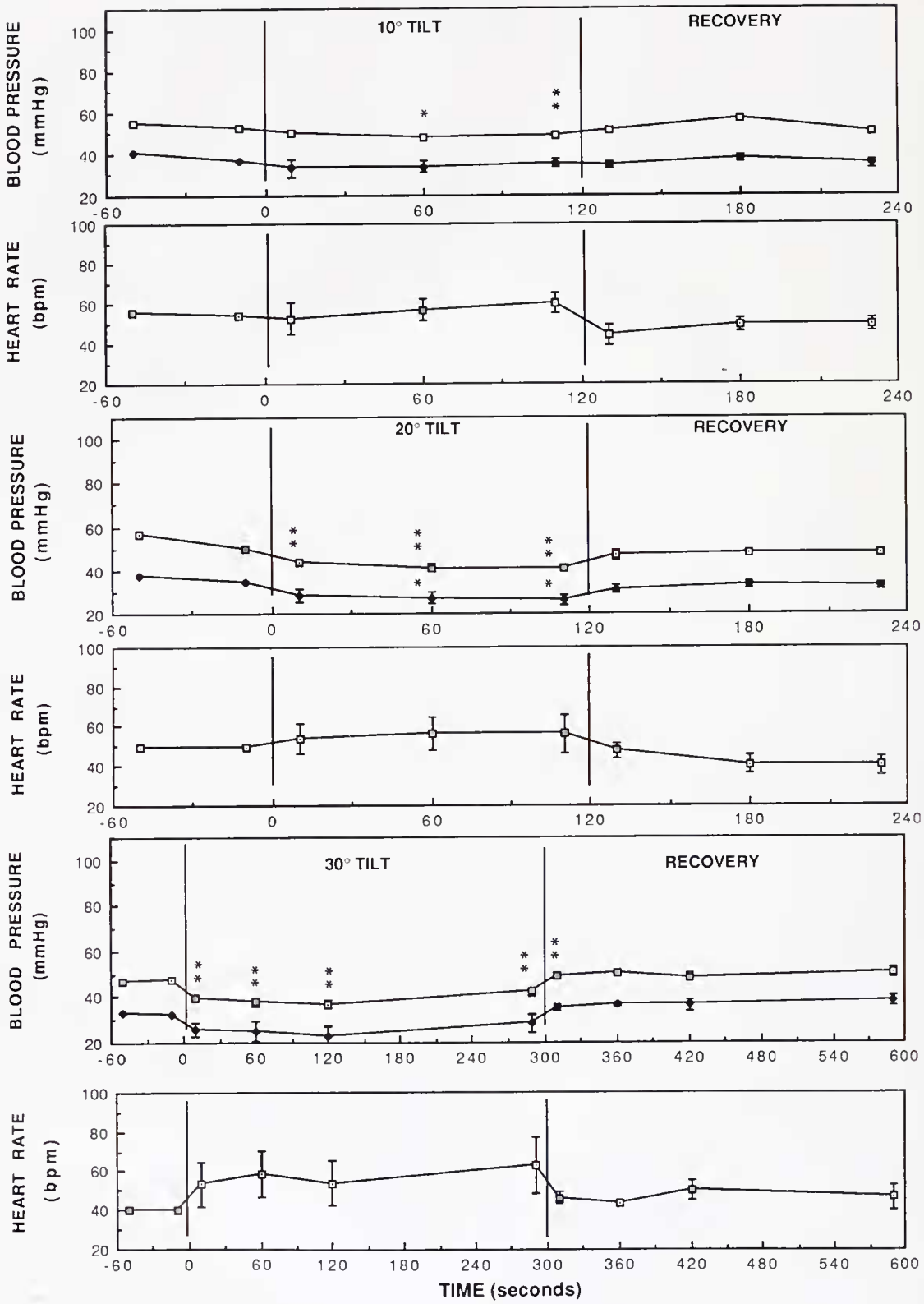

Figure 6. Head-up tilts in 5 anesthetized bluefish pretreated with 400 μg/kg of phentolamine mesylate. Ten and 20° tilts were maintained for 2 min, and the 30° tilt for 5 min.

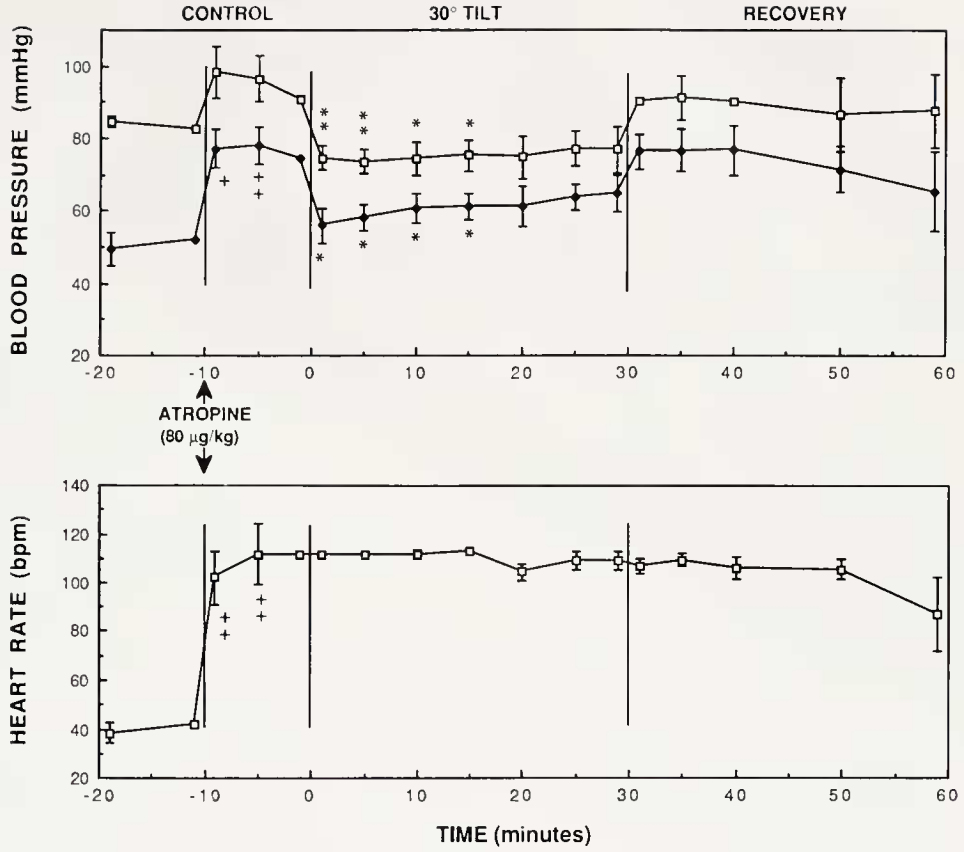

Figure 7. Atropine sulfate (80 μg/kg) was given 10 min prior to a 30° head-up tilt for 30 min in 5 anesthetized bluefish.

1–2 s in heart rate. These proved to be mediated through the vagus, implying a balance between sympathetic and parasympathetic reflex systems while the fish was at rest on the V-board. If blood pressure and heart rate were elevated due to stressful conditions, in the unanesthetized fish it may be expected that these parameters would fall over time. We observed no such reduction over time in heart rate and blood pressure.

Some general anesthetics in mammals have been implicated in resetting the baroreceptor "set point" (Marshall and Wollman, 1985). We found that although the initial blood pressure was considerably lower in anesthetized fish (Fig. 3), the response to head-up tilting was similar to unanesthetized animals (Fig. 2). In addition, anesthetized fish were able to maintain pulse pressure during the 30-min tilt (Fig. 4). We conducted the majority of tilts in anesthetized fish to allow comparison to data obtained following spinal cord transection with or without vagotomy.

Unanesthetized bluefish tolerated head-up tilts well with a tachycardia, minimal drop in blood pressure, and maintenance of pulse pressure. This response is not unlike the response described in unanesthetized humans during head-up tilts (Bie *et al.*, 1986) and lower body negative pressure (Blomquist and Stone, 1983). In mammals, this tolerance for gravity represents an integrated response due to reduced vagal outflow, peripheral sympathetic mediated arteriolar constriction and, at times, contraction of lower limb musculature to facilitate venous return to the heart (Abboud and Thames, 1983). To test the need for each of these response systems, we proceeded with experiments using sympathetic or parasympathetic pharmacologic blockade or transections of the spinal cord with and without vagotomy (Fig. 11).

The antagonist phentolamine has become a useful means of blocking alpha adrenergic mediated vasoconstriction in fish (Nilsson, 1983; Smith *et al.*, 1985; Ogilvy *et al.*, 1988). After administration of 400 μg/kg of phentolamine mesylate intra-arterially, there was a significant reduction in blood pressure (Fig. 5). During head-up tilting to 30°, there was a further significant reduction in systolic blood pressure during the first 20 min of tilting, compared to the value 1 min prior to tilt. This fall in blood pressure suggests that vasoconstriction is important in helping to maintain the blood pressure during passive head-up tilting. The tachycardia observed during

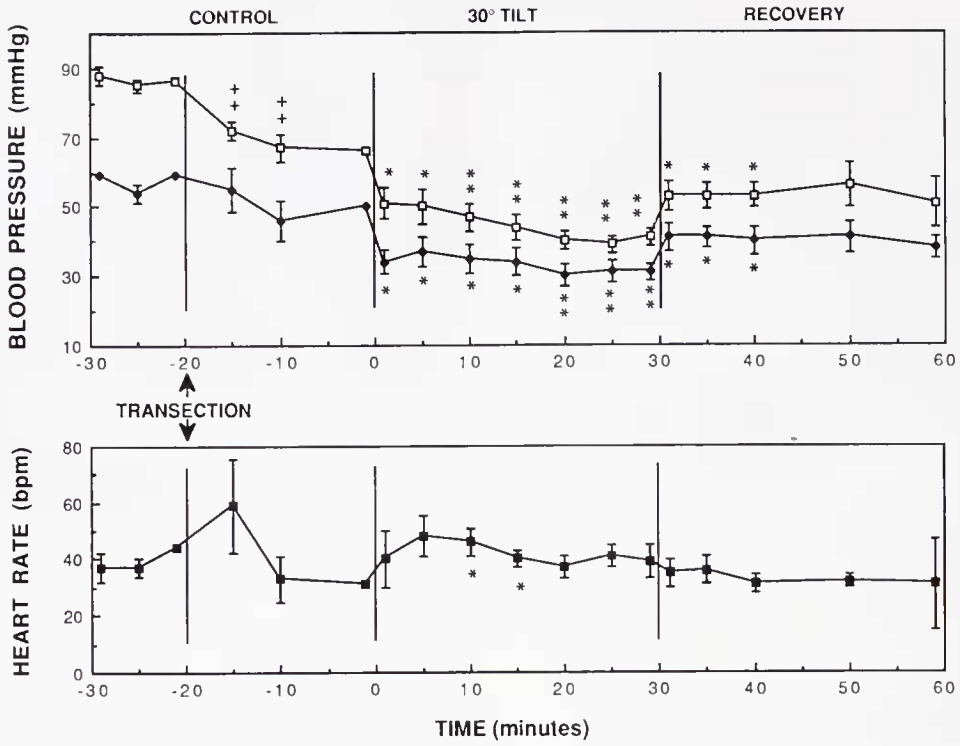

Figure 8. Transection of the spinal cord 3 mm caudal to the obex 20 min prior to a 30° head-up tilt for 30 min in 5 anesthetized bluefish.

tilts in phentolamine pretreated fish is higher than that seen in untreated fish, while pre-tilt heart rates are comparable in each of these groups of animals. The increased tachycardia in tilts with phentolamine may reflect a higher amount of baroreceptor feedback and subsequent increased suppression of vagal efferent discharge (Aboud and Thames, 1983).

The tachycardia which occurs during tilts appears to provide the fish with a useful means of attempting to maintain cardiac output (Fig. 11). This tachycardia is typical in humans in response to tilts (Bie *et al.*, 1986). We used atropine sulfate to fix the pulse at a rapid rate. As noted in results, 80 μg/kg of atropine sulfate produced a significant increase in heart rate and in blood pressure within 1 min after administration, in horizontal fish. Increased blood pressure while horizontal is presumably related to the increased cardiac output secondary to a higher heart rate. Venous return in the horizontal position is sufficient to keep up with the increased cardiac output. In the tilted position, venous return, and hence blood pressure, is diminished. Heart rate during tilts was unchanged from pre-tilt values and blood pressure fell significantly (Fig. 7). Although blood pressure was reduced, it did not approximate hypotensive levels, and as a result fish tolerated 30 min of 30° head-up tilting without any untoward effects. They swam well when returned to the holding tank. In atropine-treated fish, sympathetic arteriolar vasoconstriction as well as lower body muscle contractions can still occur, each of which contributes to an increased systemic vascular resistance and an increase in blood pressure and venous return (Fig. 11).

The spinal cord was transected 3 mm caudal to the obex to abolish both muscle tone and descending sympathetic innervation. As noted earlier, muscular contractions in our bluefish were associated with transient elevations in blood pressure (Fig. 10). This same type of behavior has been described in the black rat snake subjected to hemorrhage (Lillywhite and Smith, 1981). In these snakes wiggling movements during hemorrhage increased central venous pressure and produced a sustained increase in aortic pressure. Following spinal cord transection, bluefish became flaccid below the level of transection and were still able to make normal mouth and gill breathing motions.

Spinal cord transection with preservation of vagal fibers produced an initial tachycardia followed by a bradycardia. This type of response is typical in humans with acute complete cervical spinal cord injury where bradyarrhythmias often require atropine to prevent cardiac arrest (Lehman *et al.*, 1987). For comparison, the smooth dogfish is thought to have a less well developed cardiovascular reflex system than teleosts with relatively little resting vasomotor tone (Nilsson, 1983). These chondrichthyes tolerate total spinal cord transection with re-

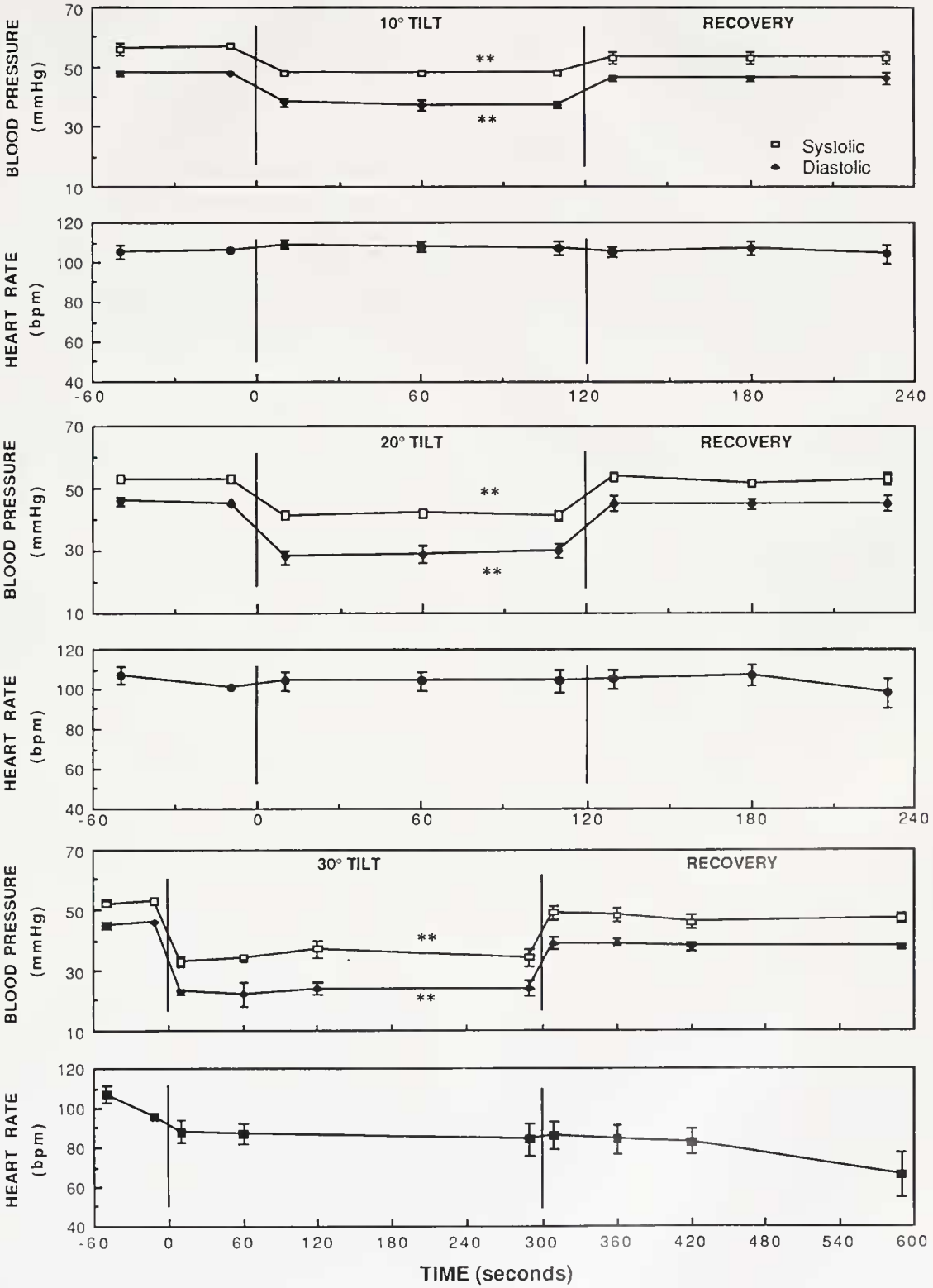

Figure 9. Blood pressure and heart rate response in 4 anesthetized bluefish, tilted 10° and 20° head-up for 2 min or 30° for 5 min, following spinal cord transection combined with bilateral vagotomy. The blood pressures during tilts are significantly lower at a level of $P < 0.001$ to $P < 0.05$ for all points during tilt compared to paired values 10 s prior to tilting.

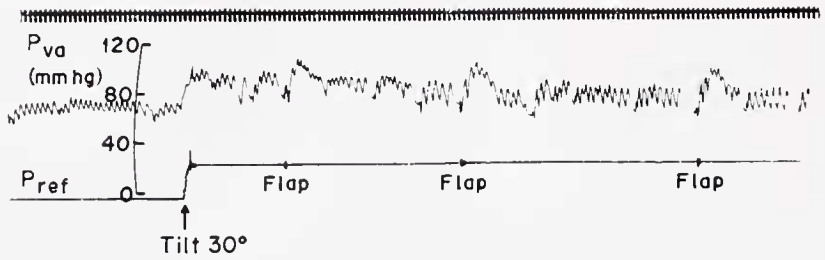

Figure 10 Typical ventral aorta blood pressure (P_{va}) response to a 30° head-up tilt in an unanesthetized bluefish pretreated with 200 μg/kg of phentolamine mesylate. Calibrations for blood pressure (in mmHg) and time marks (in seconds) are shown. The lower recording (P_{ref}) is from the reference tube set on the V-board at the level of the ventral aorta. The effective blood pressure is P_{va} minus P_{ref}. Contractions of the lower body musculature produced movements of the V-board and were recorded on the reference gauge and labelled as "flaps." Blood pressure rose in association with these movements.

moval of 1 cm of cord without any apparent change in resting heart rate or blood pressure (Opdyke *et al.*, 1972; Holcombe *et al.*, 1980). As shown in Table II, spinal cord transection without subsequent tilting produced an initial fall in blood pressure over 20 min followed by a stabilization of pressure 20 to 80 min after transection. As noted earlier, three of these four fish died of cardiac standstill, which occured with little forewarning. This was presumably based on unopposed vagal afferent input to the heart. In one fish given atropine 10 min after spinal cord transection, no pauses in heart rate were observed. In addition, in several fish where long (2 to 4 s) pauses in heart rate were observed, administration of atropine increased the heart rate and prevented subsequent episodes of severe bradycardia (Ogilvy, Fox, and DuBois, unpub. obs.).

In 5 bluefish tilted to 30° after spinal cord transection (without vagotomy), blood pressure fell despite an increase in heart rate. Theoretically, afferent impulses from the baroreceptors would still convey information to cardiovascular regulatory centers and subsequent diminished vagal output (increase in heart rate) could occur

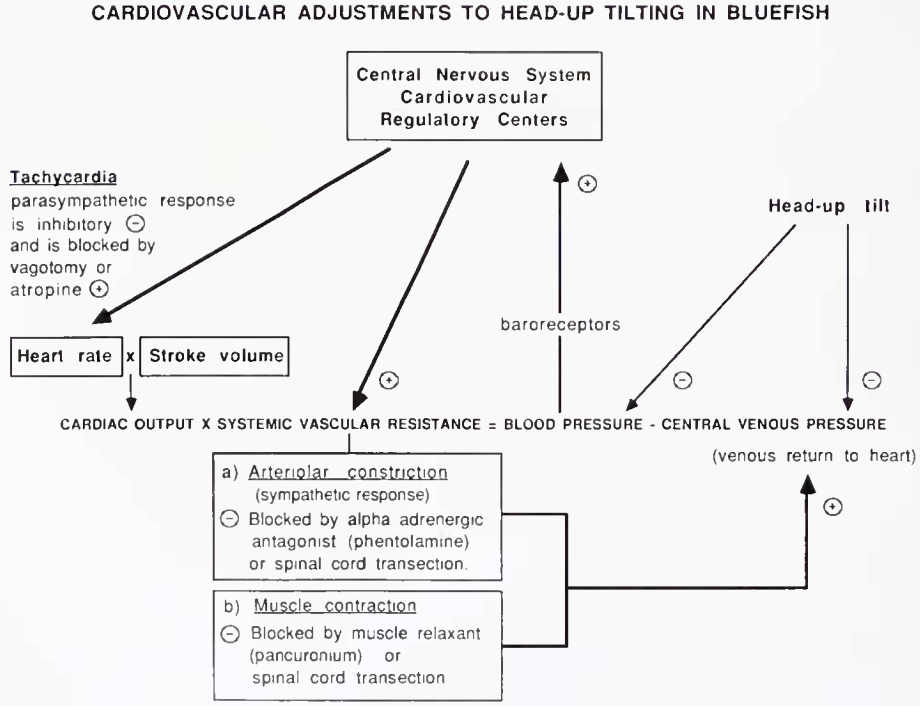

Figure 11. Summary of cardiovascular changes which occur in bluefish in response to head-up tilting based on experiments conducted using surgical lesions or pharmacologic blockade as indicated. See text for details.

(Fig. 11). Transection of the spinal cord disrupts sympathetic descending fibers as well as corticospinal tract fibers. Thus, constriction of peripheral arterioles or contraction of lower body musculature, two actions which could potentially increase systemic vascular resistance and contribute to maintaining blood pressure during tilts, are blocked. When muscle contractions were abolished in one fish by administration of the neuromuscular blocking agent pancuronium bromide, blood pressure fell during tilting.

Spinal cord transection was combined with vagotomy to abolish vagal control on the heart as well as muscular contraction of the lower body and descending sympathetic fibers within the spinal cord. In this group of fish, the fall in blood pressure was significant during 10°, 20°, and 30° tilts (Fig. 9). The fact that blood pressure fell and there was no change in heart rate implies total disruption of the usual reflex mechanisms which compensate blood pressure during head-up tilts.

Bluefish appear to have a well-developed set of cardiovascular reflex responses including both sympathetic and parasympathetic components. A summary of how bluefish respond to the physiological stress of head-up tilting is provided in Figure 11. During a head-up tilt, there is presumably diminished venous return (as in mammals) as well as initial alteration of blood pressure. The blood pressure change is relayed to central nervous system cardiovascular regulatory centers via afferent nerve fibers from baroreceptors located in the gill arches. Several changes occur in response to this information, the net effect of which is to maintain blood pressure and continue perfusion to vital organs. There is a tachycardia mediated by inhibition of the tonic vagal influence on the heart. This parasympathetic component of the response system can be altered by surgical vagotomy or by administration of atropine. Increased sympathetic tone causes alpha-receptor-mediated arteriolar constriction which acts to increase systemic vascular resistance. This arm of the response system can be blocked with phentolamine or by disrupting the sympathetic fibers surgically as they descend in the spinal cord. One further means of increasing systemic vascular resistance in the fish used in this study appears to be contraction of the body musculature. This should theoretically also assist venous return. This body contraction mechanism can be abolished with a neuromuscular junction blocking agent (pancuronium) or by transecting corticospinal tract fibers in the spinal cord. A similar degree of complexity of vasomotor control has been described in rainbow trout at rest (Wood and Shelton, 1980), in codfish at rest (Smith *et al.*, 1985; Axelsson, 1988) and in codfish during exercise (Axelsson and Nilsson, 1986; Axelsson, 1988). Adrenergic vascular innervation has been demonstrated in a variety of teleosts (Burnstock 1969; Nilsson, 1976, 1983). The presence of adrenergic vascular innervation implies that active vasoconstriction would be mediated through this innervation. This mechanism of response is different from that observed in dogfish where adrenergic vasoconstriction is stimulated by increases in circulating catecholamines which are liberated by angiotensin and not by neurogenic activity in the brain (Holcombe *et al.*, 1980).

It is interesting to speculate on whether the development of the tolerance of the circulatory system of teleosts for gravity developed simultaneously with that of land vertebrates, or preceded it (DuBois, 1988). Dogfish do not possess this tolerance, suggesting that the evolution of this trait occurred in Osteichthyes but not Chondrichthyes. In previous work, we have found that bluefish can accelerate with a force of 3G, and have body structures that sustain the compression caused by thrust of the tail against the inertial force of the body during rapid acceleration. The body structure also has to withstand the Pitot pressure on the front surface of the body and Bernoulli negative pressure on the "shoulder," just before the widest part of the body, while swimming through the water (DuBois *et al.*, 1976). These forces associated with swimming create circulatory transmural pressures that presumably might demand vascular responses such as those found in this study. Swimming requires an increase of cardiac output, and this increase is mediated through reflex control of the circulation.

Acknowledgments

The fish used in this study were kept in the Marine Resources Department of the Marine Biological Laboratory, Woods Hole, Massachusetts. We thank Mr. Sherman A. Goodell for catching many of the fish. Portions of this work have been presented in preliminary form (Fox *et al.*, 1987; DuBois, 1988; Ogilvy *et al.*, 1988).

Literature Cited

Abboud, F. M., and M. D. Thames. 1983. Interactions of cardiovascular reflexes in circulatory control. Chapter 19 in *The Handbook of Physiology*, Section 2: The Cardiovascular System, *3:* Peripheral Circulation and Organ Blood Flow, part 2, J. T. Shepherd and F. M. Abboud, eds. American Physiological Society, Bethesda, MD.

Axelsson, M. 1988. The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J. Exp. Biol.* **137:** 287–303.

Axelsson, M., and S. Nilsson. 1986. Blood pressure control during exercise in the Atlantic cod, *Gadus morhua*. *J. Exp. Biol.* **126:** 225–236.

Bie, P., N. H. Secher, A. Astrup, and J. Warberg. 1986. Cardiovascular and endocrine responses to head-up tilt and vasopressin infusion in humans. *Am. J. Physiol.* **251:** R735–R741.

Blomquist, C. G., and H. L. Stone. 1983. Cardiovascular adjustments to gravitational stress. Chapter 28 in *The Handbook of Physiology*, Section 2: The Cardiovascular System, *3:* Peripheral Circulation

and Organ Blood Flow, part 2, J. T. Shepherd and F. M. Abboud, eds. American Physiological Society, Bethesda, MD.

Burnstock, G. 1969. Evolution of autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacol. Rev.* **21:** 247–324.

DuBois, A. B. 1988. Evolution of gravitational tolerance in the sea. *The Physiologist* **31**(1): S98–S101.

DuBois, A. B., G. A. Cavagna, and R. S. Fox. 1976. Locomotion of bluefish. *J. Exp. Zool.* **195:** 223–235.

Fox, S. H., C. S. Ogilvy, and A. B. DuBois. 1987. Transection of the spinal cord near the obex abolishes cardiovascular compensation for gravity in bluefish (*Pomatomus saltatrix*). *Biol. Bull.* **173:** 422 (abstr.).

Fox, S. H., C. S. Ogilvy, and A. B. DuBois. 1988. Circulatory responses of bluefish to epinephrine, phentolamine, and atropine. *Biol. Bull.* **175:** 305 (abstr.).

Holcombe, R. F., D. W. Wilde, and D. F. Opdyke. 1980. Vasomotor tone in the dogfish shark. *Comp. Biochem. Physiol.* **65A:** 135–138.

Houston, A. H., J. A. Madden, R. H. Woods, and H. M. Miles. 1971. Some physiological effects of handling and tricaine methanesulphonate anaesthetization upon the brook trout, *Salvelinus fontinalis*. *J. Fish. Biol. Canada* **28:** 625–633.

Lehmann, K. G., T. G. Lane, J. M. Piepmeier, and W. P. Batsford. 1987. Cardiovascular abnormalities accompanying acute spinal cord injury in humans: incidence, time course and severity. *J. Am. Coll. Cardiol.* **10:** 46–52.

Lillywhite, H. B., and L. H. Smith. 1981. Haemodynamic responses to haemorrhage in the snake, *Elaphe obsoleta obsoleta*. *J. Exp. Biol.* **94:** 275–283.

Marshall, B. E., and H. Wollman. 1985. General anesthetics. Pp. 276–301 in *The Pharmacologic Basis of Therapeutics*, A. G. Goodman *et al.*, eds. 7th ed., MacMillan, New York.

Nilsson, S. 1976. Fluorescent histochemistry and cholinesterase staining of sympathetic ganglia in a teleost, *Gadus morrhua*. *Acta. Zool.* **57:** 69–77.

Nilsson, S. 1983. *Autonomic Nerve Function in the Vertebrates.* Springer-Verlag, Berlin, New York, Heidelberg.

Ogilvy, C. S., and A. B. DuBois. 1982. Effect of tilting on blood pressure and interstitial fluid pressures of bluefish and smooth dogfish. *Am. J. Physiol.* **242:** R70–R76.

Ogilvy, C. S., P. G. Tremml, and A. B. DuBois. 1988. Pressor and hemodiltion responses compensate for acute hemorrhage in bluefish. *Comp. Biochem. Physiol.* **A91:** 807–813.

Ogilvy, C. S., S. H. Fox, and A. B. DuBois. 1988. Physiologic mechanisms governing the cardiovascular compensation to gravity in bluefish. *Biol. Bull.* **175:** 308 (abstr.).

Opdyke, D. F., J. R. McGreehan, S. Messing, and N. E. Opdyke. 1972. Cardiovascular responses to spinal cord stimulation and autonomically active drugs in *Squalus acanthias*. *Comp. Biochem. Physiol.* **A42:** 611–620.

Priede, I. G. 1974. The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. Exp. Biol.* **60:** 305–319.

Smith, D. G., S. Nilsson, I. Wahlquist, and B.-M. Eriksson. 1985. Nervous control of the blood pressure in the Atlantic cod, *Gadus morhua*. *J Exp. Biol.* **117:** 335–347.

Wood, C. M., and G. Shelton. 1980. The reflex control of heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage, and systemic vasomotor tone. *J Exp. Biol.* **87:** 271–284.